Brief Report: Newborn Behavior Differs with Decosahexaenoic Acid Levels in Breast Milk

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Objective To assess whether natural variations in decosahexaenoic acid (DHA) levels in breast milk would relate to infant neurobehavioral outcomes at the newborn stage following equivocal findings on infant and toddler outcomes of exposure to DHA in formula and breast milk. **Methods** Breast milk samples from $N = 20$ mothers were collected 9 days after delivery, while the Brazelton Neonatal Behavioral Assessment Scale (NBAS) was administered on the infant. Milk samples were later analyzed for fatty acids, including DHA. **Results** Pearson correlations revealed a positive association between DHA concentrations in breast milk and infants' scores on the NBAS Range of State cluster score, suggesting that DHA is related to the infant's superior ability to maintain optimal arousal. **Conclusions** These results suggest that breast milk DHA is beneficial to the neonate's neurobehavioral functioning and call for investigative attention to DHA's role in potentiating optimized intellectual functioning at later stages of development. The findings may also be interpreted as supporting formula supplementation with DHA.

Key words breast milk; DHA; fatty acids; NBAS; neonates.

Mounting evidence of linkages between breastfeeding and children's superior cognitive development (Anderson, Johnstone, & Remley, 1999; Mortensen, Michaelson, Sanders, & Reinisch, 2002) has spurred interest in components of breast milk which may account for such enhancements (Uauy & Peirano, 1999). In view of their importance to early neurological development, long-chain polyunsaturated fatty acids, particularly decosahexaenoic acid (DHA), have received much investigative attention through research using different infant formulas (Farquharson et al., 1995; Uauy et al., 1996). Randomized controlled trials have compared infants receiving formula supplemented with DHA or DHA plus arachidonic acid (AA), another fatty acid, versus controls who received unsupplemented formula. Some studies (e.g., Birch, Garfield, Hoffman, Uauay, & Birch, 2000) uncovered positive results suggesting that supplementation is advantageous to toddlers' cognitive and motor development, but others (e.g., Scott et al., 1998) reported that supplementation is *dis*advantageous to intellectual functioning, and still others (Auestad et al., 2001) found no differences. Though the overall greater weight of positive results led to recent approval for supplemented formula in the United States, clarification of these equivocal results is still needed.

Insight into DHA's contribution to breastfeeding effects might also be gleaned through inquiry using human milk. This avenue has received less attention, possibly because of early comparisons between breast-fed infants (who received DHA naturally via breast milk) versus formula-fed infants (who were not exposed to DHA) which were unprofitable because effects owing to feeding method were often overshadowed by effects stemming from differences between breast- versus bottle-feeding mothers' educational and social backgrounds (Gibson & Makrides, 2001). To overcome such confounds, newer works have avoided comparisons with formula-fed infants and have instead focused on natural

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variations in breast milk levels of DHA and their associations with infant neurodevelopment, typically assessed on tests of visual functioning. These have regularly reported that DHA levels in breast milk depend on maternal diet. Some have also found that higher levels of milk DHA predict improved visual acuity in infants (e.g., Jorgensen, Hernell, Hughes, & Michaelsen, 2001), whereas others (e.g., Gibson, Neumann, & Makrides, 1997; Krasevec, Jones, Cabrera-Hernandez, Mayer, & Connor, 2002) have found no evidence of any benefits to infants' cognitive or visual development. This study extends work on infant functioning in relation to milk DHA by exploring infants at the newborn stage. Because social interaction is still limited at this early point in development, one potential source of confounds is minimized. This early stage permitted assessments to be conducted using the Brazelton Neonatal Behavioral Assessment Scale (NBAS; Brazelton & Nugent, 1995), an instrument which taps a broad range of neurobehavioral responses. In addition, to help control for the possibility that milk components other than DHA might also contribute to infant outcomes (Voigt et al., 2002), we took into account fatty acids other than DHA. In line with the weight of evidence favoring formula supplementation with DHA, we hypothesized that higher concentrations of DHA in breast milk would predict infants' superior performance on the NBAS.

Method

Participants

Participants were $N = 20$ breastfeeding dyads. Mothers of healthy, full-term infants were recruited at the maternity unit of a university hospital shortly after delivery by trained researchers who obtained consent to take part in research on neonatal behavior. Exclusion criteria included logistics that would interfere with data collection, such as living outside city limits, drug or alcohol abuse, or use of medication. Of 81 women invited to participate, 16.44% refused because of concern with the requirement to provide a milk sample, and 17.89% mentioned logistical problems. Within a week of discharge, mothers were contacted by an appointment clerk who screened out mothers who were no longer breastfeeding exclusively. This resulted in a further drop of 60% in the subject pool. Finally, one sample was considered an outlier. Demographics on the $N = 20$ participating dyads are presented in Table I.

Table I. Characteristics of Participants

	M (SD)
Maternal characteristics	
Age	20.45 (1.88)
Socioeconomic status	3.80 (0.70)
Years of schooling	12.45 (1.36)
Length of hospital stay (days)	2.75 (0.80)
Obstetric Complication Scale score	98.35 (11.30)
Postnatal Factors Scale score	103.75 (7.13)
Primiparous (%)	65
Ethnicity (%)	
Caucasian	70
African American	5
Hispanic	25
Infant characteristics	
Male (%)	60
Apgar score of 8–10 at 1 min (%)	95
Birthweight (g)	3214.50 (456.00)
Length (cm)	50.59 (3.49)
Gestational age (weeks)	39.10 (0.85)
Age at testing (days)	9.00 (0.97)

Measures

Demographic Information

These questionnaires addressed maternal age, parity, ethnicity, and education. Scores for socioeconomic status (SES) were derived from participants' family income and education, yielding values ranging from 1 to 6, with higher scores signifying lower SES (Hollingshead, 1975).

Obstetric Complications Scale and Postnatal Scale (Littman & Parmelee, 1978)

The 41-item Obstetric Complications Scale (OCS) and the 10-item Postnatal Scale (PNS) quantify obstetric and perinatal complications. Items are rated by medical chart review. Higher scores signify more optimal outcomes, and a score of 100 is normative. The charts were also accessed separately for information, such as birthweight, gestational age, and Apgars.

NBAS (Brazelton & Nugent, 1995)

This examiner administered assessment of newborn neurobehavioral functioning includes 28 items, scored on 9-point scales, which are then subjected to clustering techniques, yielding summary scores for: habituation, orientation, motor, range of state, regulation of state, and autonomic stability. Additional summary scores index atypical responses, including abnormal reflexes, excitability, and depression (Lester, Freier, & LaGasse, 1995).

Fatty Acid Analysis

Total lipids were extracted from the breast milk using 2:1:1 methanol : chloroform : sample ratio. Methanolic HCl and dimethoxypropane were added to the lipid residue, mixed well, and incubated at room temperature overnight. The remaining residue after a *n*-hexane extraction was resuspended in *n*-hexane for injection into the gas chromatograph (Shimadzu, Columbia, MD) at an injection temperature of 200°C and detection temperature of 250°C. Peaks were identified by retention times relative to authentic standards. Fatty acids are reported as a percentage of the total lipid concentration. All assays were conducted in triplicate, and means were used.

Procedure

Home visits took place 9 (*SD* = .97) days after delivery, 1–2 h after an infant's last feeding, in late morning or early afternoon time slots, and following a period of at least 2 h during which time mothers had not breastfed their infants. This scheduling provided adequate time in which breastfeeding could begin to become established and helped control for diurnal fluctuations in fatty acid levels in milk (Lauritzen, Jorgensen, Hansen, & Michaelsen, 2002; Lawrence & Lawrence, 1999). After verifying that the infant was in good health, and securing a quiet location, the examiner (S.C. or Y.M.) administered the NBAS. The examiners had been trained on the NBAS by the first author (Hart, Field, & Nearing, 1998). Inter-rater reliability between the three researchers had been achieved at 94% (where agreements were calculated as item scores ± 1 point/total score) on $N = 20$ infants before the study. Thereafter, this level of inter-rater reliability was reevaluated on seven infants, representing 33.33% of the entire sample, and maintained at 95%. During the NBAS administration, the mother collected a milk sample. The entire supply of milk from one breast was collected into a sterile tube connected to an electric breast pump (Medela, McHenry, IL). Tubes were labeled with the subject number, kept on ice, and protected from light during transport to the laboratory where they were immediately frozen at –80°C until analysis by an author (C.K.).

Results

Preliminary analyses revealed that breast milk DHA levels were uncorrelated with maternal characteristics, including age ($r = .33$, $p < .15$), education ($r = .19$, $p < .42$), SES ($r = .20$, $p < .42$), and obstetric complications ($r = .28$, $p < .29$), or infant characteristics, including age on day of testing ($r = -.18$, $p < .44$), gestational age ($r = .31$, $p < .19$), birthweight ($r = .09$, $p < .68$), and postnatal factors ($r = .25$, $p < .28$). Similarly, chi-square analyses revealed that high versus low (using median split) levels of breast milk DHA were unrelated to maternal parity ($\chi^2 = 1.98$, $p = .16$), ethnicity ($\chi^2 = 1.49$, $p = .47$), and infant sex ($\chi^2 = 0.83$, $p = .36$). Analyses of fatty acids revealed a mean value for DHA of 0.08% of total lipids (Table II). This level in milk at 9 days postpartum is lower than levels which have been reported by others, such as Guesnet and associates (Guesnet, Antoine, Rochette de Lempdes, Galent, & Durand, 1993), who reported values of 0.27 and 0.15% at 3 and 15 days postpartum respectively, and Makrides and associates (Makrides, Simmer, Neumann, & Gibson, 1995) who found values of 0.16% at 6 weeks of postpartum. Values of fatty acids and NBAS cluster scores are presented in Table II.

To analyze associations between infants' fatty acids in milk and NBAS scores, Pearson correlations were conducted using the more stringent $p < .01$ level of significance to help control for the number of analyses being conducted. These revealed a positive association between DHA and the range of state cluster score $r = .57$, $p < .01$. Further inquiry into the four items which comprise this cluster revealed that the association stemmed from DHA's significant association with one item, lability of state, and is illustrated in Fig. 1. Inspection of the raw scores for this item revealed that in comparison with infants who were exposed to higher levels of milk DHA, infants who were exposed to lower levels of milk DHA changed state of arousal at both greater and lesser

Table II. Mean Values Obtained Through Analyses of Fatty Acids in Breast Milk, and Brazelton Neonatal Behavioral Assessment Scale (NBAS) Cluster Scores Obtained by Infants

	M (SD)
Fatty acid values	
Myristic	6.43 (2.10)
Palmitic	21.44 (1.98)
Palmitoleic	3.88 (0.88)
Stearic	4.88 (0.90)
Oleic	33.74 (4.11)
Linoleic	13.67 (2.43)
α-Linoleic	0.91 (0.28)
Arachidonic	0.41 (0.10)
Eicosasapentaenoic	0.05 (0.09)
Docosapentaenoic	0.06 (0.06)
Decosahexaenoic	0.08 (0.03)
NBAS cluster scores	
Habituation	4.75 (3.05)
Orientation	5.17 (2.12)
Motor	4.67 (0.69)
Range of state	4.07 (0.61)
Regulation of state	4.48 (1.34)
Autonomic stability	6.63 (1.01)
Abnormal reflexes[a]	3.45 (2.06)
Excitability[a]	1.60 (1.35)
Depression[a]	1.70 (1.81)

[a]Higher scores denote poorer performance.

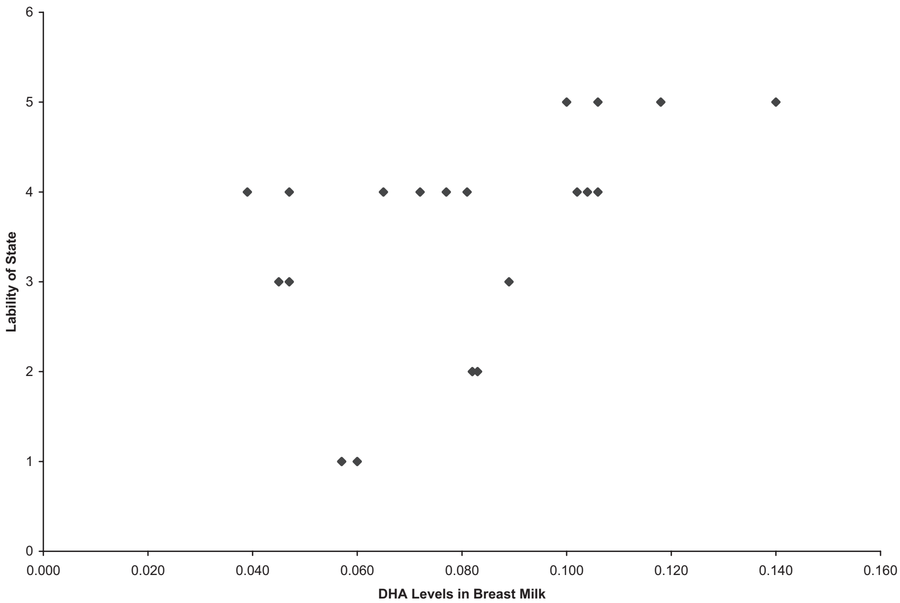

Figure 1. Scatter plot illustrating association between decosahexaenoic acid (DHA) levels in breast milk and infant performance on the Brazelton Neonatal Behavioral Assessment Scale (NBAS) lability of state item.

frequencies. None of the other fatty acids were related to any of the NBAS cluster scores.

Discussion

In support of our hypothesis that higher concentrations of DHA in breast milk would be associated with infants' optimized performance on the NBAS, findings of this study identified superior arousal in infants of mothers whose milk contained greater concentrations of DHA. Because breast milk, unlike formula, is highly variable, we cannot rule out the possibility that this association was mediated by coinciding influences. The possibility that another fatty acid could have contributed to infant performance on the NBAS is diminished by the fact that our inquiry into linkages of this nature yielded no significant results. Nor is it likely that social influences played a contributing role since all of the infants were being breastfed and, at only 9 days of age, exposure to social influences had been minimal. Also, analyses of demographic variables revealed that mothers whose milk was high in DHA did not differ from those with lower concentrations. Thus, the results point to the importance of DHA itself and call for further attention to DHA in breast milk. In particular, work must address earlier exposure to DHA *in utero*, and the extent to which pre- and perinatal exposures account for optimized neurobehavioral functioning which has been documented in breast-fed newborns (Hart, Boylan, Carroll, Musick, & Lampe, 2003). Longitudinal studies must also address the sequelae of early exposure to DHA in breast milk, including the possibility that DHA's contribution to superior arousal during infancy might underlie later advantages to children's intellectual functioning (Anderson et al., 1999; Mortensen et al., 2002). An unexpected, and disturbing, finding which emerged from this study was DHA's low level in breast milk. This may have been attributable to mothers' substandard dietary intakes since earlier research (Kuratko, Hart, Border, & Boylan, 1999) using similar samples of lactating women had documented low consumption of fruits, vegetables, and fish. The importance of DHA in maternal diet has been highlighted by findings that benefits of breastfeeding to children's cognitive attainments were augmented, if the maternal diet during pregnancy and lactation had been supplemented with DHA in cod liver oil (Helland, Smith, Saarem, Saugstad, & Drevon, 2003). Thus, it appears clear that although many questions await investigative attention, the clinician's continuing emphasis on maternal nutrition throughout the period of lactation is of utmost importance.

This study's findings are also of importance to researchers and clinicians with interest in infant formulas. To enhance the rigor of their studies, newer trials comparing infants receiving supplemented versus unsupplemented formula (e.g., Auestad et al., 2001; Birch et al., 2000; Scott et al., 1998) often also include a group of breastfed infants as a reference, or "gold standard group" (Birch et al., 2000). Based on our finding that variations in breast milk DHA levels have implications for infant neurodevelopment, it appears that some attention to natural variability in breast milk levels of DHA within this reference group is necessary to derive any useful information from the inclusion of these infants. Finally, the findings may be of some use to clinicians who are sought out by formula-feeding mothers for advice on whether to provide their infants with formula which has been supplemented with DHA. Given the equivocal findings of research on formula supplementation and the unprecedented and ambivalent situation in which formula supplementation has been made available to consumers on an optional basis, pediatricians may be willing to consider findings of research using a different approach, which focused on DHA in milk rather than formula. In this case, the association between breast milk DHA and newborn infants' optimized arousal may cautiously be taken as evidence in favor of formula supplementation.

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